Published in final edited form as:

Respir Physiol Neurobiol. 2012 August 15; 183(2): 159–165. doi:10.1016/j.resp.2012.06.004.

Hypoxia Induced Changes in Lung Fluid Balance in Humans is Associated with Beta-2 Adrenergic Receptor Density on Lymphocytes

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Abstract

Background—Previous studies have demonstrated an important role for beta-2 adrenergic receptors (β_2 AR) in lung fluid clearance. The purpose of this investigation was to examine the relationship between β_2 AR density on lymphocytes and indices of lung water in healthy humans exposed to ~17hr of hypoxia (F_{IO_2} 12.5% in a hypoxia tent).

Methods—Thirteen adults (mean $\pm$ SEM; age=31 $\pm$ 3yr, BMI=24 $\pm$ 1 kg/m², V_{O_2} Peak=40 $\pm$ 2 ml/kg/min) participated. Pulmonary function, CT derived lung tissue volume (V_{tis} -tissue, blood & water), lung diffusing capacity for carbon monoxide (D_{CO}) and nitric oxide (D_{NO}), alveolar-capillary conductance (D_M), pulmonary capillary blood volume (V_c) and lung water (CT V_{tis} – V_c) were assessed before and after ~17hr normobaric hypoxia (F_{IO_2} 12.5%). β_2 AR density on lymphocytes was measured via radioligand binding. Arterial oxygen saturation (Sa_{O_2}), cardiac output (Q), right ventricular systolic pressure (RVSP) and blood pressure (BP) were also assessed.

Results—After 17hr hypoxia, Sa_{O_2} decreased from 97 $\pm$ 1 (normoxia) to 82 $\pm$ 4% and RVSP increased from 15 $\pm$ 9 (normoxia) to 28 $\pm$ 4mmHg ($p<0.05$) with little change in Q or BP. V_c and D_M both increased with hypoxia with a small increase in D_M/V_c ratio ($p>0.05$). CT V_{tis} decreased and lung water was estimated to decline 7 $\pm$ 13%, respectively. β_2 AR density averaged 1497 $\pm$ 187 receptors/lymphocyte and increased 21 $\pm$ 34% with hypoxia (range –31 to +86%). The post-hypoxia increase in β_2 AR density was significantly related to the reduction in lung water ($r=-0.64$, $p<0.05$), with the subjects with the greatest increase in density demonstrating the largest decline in lung water.

Conclusions—Lung water decreases with 17hr normobaric hypoxia are associated with changes in beta adrenergic receptor density on lymphocytes in healthy adults.

Keywords

Lung water; edema; altitude physiology; pulmonary congestion

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1. INTRODUCTION

Fluid flux between the pulmonary capillaries, the interstitial space, and the pulmonary lymphatics follow the forces described by Starling (Starling, 1896). If the hydrostatic pressure of the pulmonary capillaries is greater than the hydrostatic pressure of the interstitium, there will be an increased fluid transfer into the interstitial space. The pulmonary lymphatics act to remove fluid from the interstitial space which reduces the interstitial hydrostatic pressures and, therefore, plays a role in preventing excessive alveolar fluid accumulation (Lauweryns and Baert, 1977; Mahe et al., 1991). Fluid in the alveoli can increase if interstitial fluid accumulation becomes greater than interstitial removal. In addition to these factors regulating lung water, epithelial sodium channels (ENaC) on the apical portion of type II alveolar cells serve to actively clear fluid from the alveoli (Johnson et al., 2006; Matalon et al., 2002; Olivier et al., 2002).

The beta-2 adrenergic receptors (β_2 AR) are found throughout the lungs, in the airways, the pulmonary vasculature, and the pulmonary lymphatic tissue. Stimulation of the β_2 AR causes an increase in cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) which leads to an increase in the number of ENaC on the apical portion of both type-I and type-II alveolar cells, and the probability that an ENaC is open (Chen et al., 2001; Eaton et al., 2004). In addition, increases in cAMP secondary to β_2 AR stimulation leads to smooth muscle relaxation of the pulmonary lymphatics, which may also play a role in lung fluid balance (Ikomi et al., 1991; Mahe et al., 1991). In mice, over-expression of the β_2 ARs stimulates fluid clearance from the apical side of the alveoli (Factor et al., 2002). In addition, β -agonists increase alveolar fluid clearance in animals and reduce clinical symptoms of high altitude pulmonary edema (HAPE) in humans (Davis et al., 1979; Sartori et al., 2002). Moreover, we have previously demonstrated differences in lung fluid accumulation according to β_2 AR genotype (Snyder et al., 2006c). Collectively, these data identify an important role for the β_2 AR on lung fluid regulation in humans and animals.

It has been shown previously that changes in lymphocyte β_2 AR density and activity are similar to the changes observed in β_2 AR density and activity in the heart and lungs in response to prolonged stimulation with an exogenous agonist (when imaged using positron emission tomography scanning) (Qing et al., 1997). We have previously demonstrated that β_2 AR density on lymphocytes is related to cardiovascular function at rest and during exercise in healthy humans (Snyder et al., 2006b). The impact of acute hypoxic exposure on β_2 AR density in the lungs remains controversial. For example, Birkrant *et al.* (1991) demonstrated that hypoxia increased β -AR density in the lungs of animals. By contrast, it has been shown that prolonged hypoxia causes a reduction of β -AR density in the heart and pulmonary vasculature of mice, although this may be due primarily to a reduction in β_1 AR rather than β_2 AR (Li et al., 1996; Voelkel et al., 1981). At least one investigation has found that hypoxia results in an increase in β -AR density in the lungs of animals (Birkrant et al., 1991). Work from our laboratory and others suggests that the lungs in healthy humans and animals may lose lung water when exposed to short-term normobaric hypoxia, however, the mechanism of this reduction in lung water remains unclear (Aarseth and Karlsen, 1977; Bland et al., 1977; Sakuma et al., 2001; Snyder et al., 2006a). Thus, the purpose of this study was to examine the relationship between β_2 AR density on lymphocytes and indices of lung water in healthy humans exposed to hypoxia. Given the importance of the β_2 AR on lung fluid clearance, along with our previous work demonstrating a relationship between β_2 AR density and cardiovascular function, we hypothesized that the loss in lung water may be associated with greater β_2 AR density on lymphocytes.

2. METHODS

2.1 Subjects

The protocol was reviewed and approved by the Mayo Clinic Institutional Review Board. All participants signed informed consent before study, and all portions of the study were performed according to the Declaration of Helsinki. Thirteen healthy nonsmokers who were free from cardiopulmonary abnormalities and were not taking prescription medications for cardiovascular or lung disease participated in the study. Subject characteristics and baseline pulmonary function are shown in Table 1 and Figure 1, respectively.

2.2 Protocol Overview

The study involved two separate visits to our laboratory, including 1) a screening visit, and 2) a hypoxic exposure visit. The screening tests included baseline pulmonary function testing, an incremental cycle ergometry test to exhaustion (to rule out cardiopulmonary abnormalities), a complete blood count (to rule out anemia), and, in women, a pregnancy test. Key methods performed before and after ~17 hr hypoxic exposure included, CT imaging for measures of lung tissue volume (V_{tis}), pulmonary function testing (spirometry), along with measures of lung diffusing capacity for carbon monoxide (D_{CO}) & nitric oxide (D_{NO}) to obtain estimates of alveolar-capillary conductance (D_M), pulmonary capillary blood volume (V_c) and an estimate of lung water (tissue volume corrected for pulmonary capillary blood volume, $CT V_{tis} - V_c$), as described previously. In addition, β_2AR density on lymphocytes was determined using radioligand binding. Subjects were monitored for arterial oxygen saturation (SaO_2), cardiac output (Q), right ventricular systolic pressure (RVSP, using tricuspid regurgitant velocity, using echocardiography) and blood pressure (BP) before and during the normobaric hypoxia.

2.3 Hypoxia visit

Subjects checked into our laboratory at 1300hr for the resting hypoxic exposure visit. Pre hypoxia CT-scanning and measures of V_c , D_M , Q and spirometry were performed, and blood samples were drawn for the assessment of catecholamines, a complete blood count, and radioligand binding. On this visit, a normoxic echocardiogram was also performed for the assessment of RVSP. Upon completion of the baseline room air tests, subjects entered a hypoxic tent at 1400hr with a controlled inspired oxygen concentration ($F_{IO_2}=12.5\%$) (Colorado Altitude Training, Boulder, CO) and remained in the tent for 17 hours overnight. While in the tent, the subject's HR and SaO_2 were monitored continuously by pulse oximetry (Nellcor, Pleasanton, CA). BP, symptoms (modified Lake Louise scale), and tent gases were monitored every two hours by a nurse in the Mayo Clinic Center for Translational Sciences Activities (CTSA). Following completion of the overnight stay in the hypoxic tent (at 0700), bloods were again drawn, and a repeat echocardiogram was performed. Subjects were then connected to a mask attached to a large gas reservoir, which was connected to a portable gas tank of 12.5% O_2 gas, which allowed the subjects to maintain hypoxia while in transit to the CT scanner and physiological core laboratory. The subjects then performed repeat measures of V_c , D_M , lung density, and tissue volume, as well as spirometry while maintaining 12.5% F_{IO_2} on the CT scanner and in the laboratory.

2.4 Data collection

Measures of Pulmonary Capillary Blood Volume and Alveolar-Capillary Conductance— D_{CO} and D_{NO} were measured in triplicate before and after hypoxic exposure using a rebreath technique with gases sampled on a mass spectrometer (Perkin-Elmer, 1100) and nitric oxide (NO) analyzer (Sievers Instruments, Boulder, CO) using custom analysis software. A 5-liter rebreath bag was filled with 0.3% carbon monoxide

(C¹⁸O), 40 PPM NO (diluted immediately before the test in the bag from an 800 PPM gas mixture), and O₂ (18% for the initial breath which averaged 12% over 10 breaths). For the prehypoxia measures, the subject's alveolar O₂ was allowed to equilibrate to the low FI_{O2} used in the test gas mixture by allowing the subjects to prebreathe 12.5% O₂ for 10 breaths and then immediately switching them into the rebreathe bag containing the test gas mixture, allowing us to more accurately compare pre- and posthypoxia measures. The volume of gas used to fill the rebreathe bag was determined by the tidal volume of the subject. The subjects were coached to maintain a constant respiratory rate during the rebreathe maneuver which was controlled using a metronome set to 32 breaths per minute.

Catecholamines—Epinephrine (Epi) and norepinephrine (NE) were assessed according to methods developed in the Mayo Clinic CTSA immunochemical core laboratory and the methods of Sealey (Sealey, 1991). Our laboratory intra-assay coefficients of variation are as follows: NE 4.5% and 3.3% at 224 and 429 pg/ml and Epi 12.2% and 3.6% at 13.8 and 242 pg/ml, respectively. Interassay coefficients of variation are as follows: NE 8.2% and 6.3% at 337 and 533 pg/ml and Epi 8.5% and 6.3% at 179 and 390 pg/ml, respectively.

Pulmonary Vascular Pressures—Pulmonary arterial pressure was calculated from the tricuspid regurgitation (TR) velocity as described previously, using the equation $P=4v^2$, where P is the pressure and v is the TR velocity (Yock and Popp, 1984). The same sonographer was used for pre- and post measures. Venous injections of agitated saline were used as needed to enhance the signal for TR velocity.

Spirometry, Q and HR—The maximal expiratory flow-volume (MEFV) data (forced vital capacity, FVC, forced expiratory volume in 1 second, FEV1 and forced expiratory flow between 25 and 50% of forced vital capacity, FEF25–75%) were obtained on the same mouthpiece as the lung-diffusing capacity measures and performed in accordance with previously published American Thoracic Society guidelines (1995).

Measures of Q and HR—Cardiac output was assessed using a previously validated 8 to 10-breath acetylene (C₂H₂) rebreathe technique using the same anesthesia bag containing the diffusion mixtures with the addition of 0.7% C₂H₂ and 9% helium. During each test, HR was assessed using a 12-lead electrocardiogram.

Measures of β₂AR- Density on Lymphocytes—Blood was drawn after 15 minutes of quiet rest and lymphocytes were isolated as described by Boyum (Boyum, 1968, 1976). Immediately following the blood draw, radioligand binding was performed. Forty milliliters of blood was drawn into sodium heparin tubes and transferred to serum separator tubes for isolation of mononuclear cells (BD Biosciences, Franklin Lakes, NJ). Whole blood was then centrifugated at 3000rpm at 24°C for 25 minutes. The mononuclear cells were then transferred to a 50ml centrifugation tube following the addition of Hank's balanced salt solution (Fisher Scientific, Houston, TX) and centrifugated at 1000rpm for 8 minutes. The cell pellet was then re-suspended in Hank's solution. Triplicate samples of 5×10⁵ cells were prepared in ¹²⁵I-iodocyanopindolol (¹²⁵I-CYP, Fisher Scientific, Houston, TX) in a concentration of 160pM for receptor binding. To identify non-specific binding, half of the tubes were prepared with the addition of a cold-ligand (+/−) propranolol (Sigma, St. Louis, MO) at a vast molar excess (10μM) to displace specific binding. For receptor density, specific binding was defined as total binding minus non-specific binding.

Measures of CT-based Lung Tissue Volume and Density—All CT scans were performed on the same scanner (GE LiteSpeed spiral CT scanner, GE Healthcare). Initial slices were obtained for all scans which were 2.5mm thick with a 1.2mm overlap and then

reconstructed to 1.25mm with a 0.6mm overlap. Prior to the baseline scan a scout scan was performed to determine the location and size of the lungs. Marks were placed on the skin of the subject to indicate the anatomical location of the start of the scan and the table height was recorded to insure consistency with post-scans. The number of slices of the baseline scans was recorded in order to have consistent scans throughout the study.

Normalization of Lung Volumes during Scanning—Subjects were asked to breathe on a mouthpiece connected to a pneumotachometer which was integrated with a portable computer with custom analysis software in order to provide accurate lung volumes during the scans. During the scans, the investigators observed the breathing pattern of each subject and instructed them to the desired lung volume for the breath-hold during scanning. For the baseline scans, subjects were asked to hold their breath at two lung volumes, total lung capacity (TLC) and end expiratory lung volume (EELV). The post-hypoxia scans were performed at end-inspiratory lung volume (EILV). To obtain consistent lung density data while controlling for lung volume, the image data obtained post hypoxia were compared to values obtained by interpolating between the images obtained at TLC and EELV during baseline scanning. This scanning strategy was performed to insure accurate comparisons, because it is nearly impossible to exactly match baseline and post intervention lung volumes, while minimizing the number of scans and amount of radiation exposure to the subjects.

Lung Density and Tissue Volume Analysis Using CT—The CT images were submitted to analysis using custom image analysis software (PW2, VIDA Diagnostics, University of Iowa, Iowa City IA). The analyses were performed by a graduate student who was blinded to the condition of the subject's CT scan. The software first segmented the images to separate lung tissue from surrounding structures and the mediastinum and has been validated in sheep models (Hoffman et al., 2006). In each picture element, lung density was assumed to be a linear combination of air (Hounsfield units, HU= -1000) and lung tissue, which has density of water (HU=0). Thus, HU of -600 and -400 would represent 40% and 60% tissue, respectively. A histogram analysis of picture elements within the lung tissue was performed to obtain mean lung density in HU and tissue volume by summation among all elements in the lung fields. Lung tissue volume and lung density data obtained from baseline images were plotted against lung volume to be used for linear interpolation to obtain data against which other images were compared.

Estimation of Lung Water—Tissue volume as assessed by CT includes lung tissue, blood, and water. By combining our measures of tissue volume and Vc obtained from the lung diffusing capacity tests, we were able to estimate changes in lung water from baseline to post-saline using the following equation: $Lung\ Water = (Pst\ TV - VcPst) - (IP\ TV - VcIP)$. Where Pst TV is the given tissue volume from the CT scan post-intervention (in ml), IP TV is the interpolated tissue volume at the post-intervention lung volume (in ml) from baseline CT scans, and Vc is the pulmonary capillary blood volume in ml at each time point, Pst is post intervention, IP is baseline.

2.5 Data Analysis

All statistical comparisons were made using the SPSS statistical software package (version 11.0, Chicago, IL). Group demographics were compared using an independent samples t-test with an alpha level of 0.05 to determine statistical significance. The changes in the physiological parameters, pulmonary function, catecholamines, D_{CO} , D_M , Vc, tissue volume and lung water in response to hypoxia were compared using a repeated measures ANOVA with an alpha level of 0.05 to determine significance. All data were assessed for normality prior to the ANOVA using a Levene's test. The differences in the physiological parameters,

pulmonary function, catecholamines, D_{CO} , D_M , V_c , tissue volume and lung water between the groups at baseline and post-hypoxia were compared using an independent samples t-test (alpha level of 0.05).

3. RESULTS

Hypoxic exposure

Hypoxic exposure caused a reduction in Sa_{O_2} , an increase in heart rate (HR), right ventricular systolic pressure (RVSP), epinephrine, and norepinephrine, and no change in mean arterial pressure or systemic vascular resistance (table 2). Hypoxia also increased symptoms (i.e. headache, dizziness, etc.) as recorded through the modified Lake Louise questionnaire. In addition, there were small but significant increases in pulmonary function (FVC, FEV_1 and FEF_{25-75}) (figure 1). In agreement with our previous findings, alveolar-capillary conductance (D_M) and pulmonary capillary volume (V_c) increased from pre-to post-hypoxia; however, the ratio of D_M/V_c also increased, when expressed both as absolute values and as percent change from normoxia ($p < 0.05$, table 2), indicating that the rise in D_M was greater than that of V_c . There was also an increase in the number of β_2 ARs per lymphocyte following exposure to 17 hours of hypoxia ($p < 0.05$, figure 2).

The CT-determined V_{tis} decreased from pre- to post-hypoxia in most subjects (figure 3). When combining the drop V_{tis} with the increase in V_c determined with lung diffusion measures, we found a 45 ± 27 ml ($\sim -7\%$) decrease in estimated lung water (figure 4). When examining the association of the changes in β_2 ARs on lymphocytes with hypoxia, there was a negative correlation with the changes in lung fluid so that subjects with the greatest rise in β_2 ARs had the greatest fall in our estimate of lung water with hypoxia ($r = -0.64$, $p < 0.05$, figure 5).

4. DISCUSSION

The main findings of the present study were that short term normobaric hypoxia 1) increased alveolar-capillary conductance, even when corrected for changes in pulmonary capillary blood volume (V_c), 2) decreased CT derived measures of lung tissue volume and estimated lung water (lung tissue – V_c), 3) increased lymphocyte beta-2 adrenergic receptor density, and 4) increased maximal lung volumes and expiratory flow rates in healthy humans. The primary novel finding of the presents study (that builds on our previous work) is the observation of a significant negative relationship between the hypoxia-induced reduction in lung water and the increase in lymphocyte β_2 AR density; that is, subjects with the greatest rise in β_2 AR density had greater evidence of fluid clearance in the lungs. This study suggests that the previously-observed decrease in lung water with short-term hypoxic exposure could be, at least in part, modulated by upregulation of the β_2 ARs.

4.1 Lung Fluid Regulation in Humans

The regulation of lung fluid in humans follows the laws of Starling and is a dynamic process whereby water, electrolytes, and proteins move from the pulmonary vessels into the interstitial space. Water then moves into the airways and alveolar space through aquaporin channels (Agre, 2006; Verkman et al., 2000). In addition, water can follow Cl^- which is actively moved to the apical side of alveolar cells through several pathways including the cystic fibrosis transmembrane regulator (Puchelle et al., 1992; Taouil et al., 2003). Ions and water are actively removed from the alveolar space and back to the interstitial space primarily through the epithelial Na^+ channels (ENaC) (Hummler et al., 1996; Morris and Schafer, 2002; Olivier et al., 2002). Fluid can be removed from the interstitial space by the

lymphatic system, thus preventing excess fluid movement into the alveolar airspace (Lauweryns and Baert, 1977).

There are several clinical and environmental conditions, such as decompensated heart failure and exposure to high altitude, that challenge the ability of the lungs to clear fluid causing an accumulation of fluid in the interstitial space and alveoli that can hinder gas transfer from the alveoli to the pulmonary capillaries. Some, but not all, individuals who travel to altitude are susceptible to high-altitude pulmonary edema (Basnyat, 2003; Steinacker et al., 1998). Similarly, not all patients with decompensated heart failure develop pulmonary edema, despite similar clinical co-morbidities. Because there appear to be some individuals who are susceptible to lung fluid accumulation, it is important to determine what pathways are important in lung fluid clearance, in-vivo.

4.2 The Importance of the β_2 ARs in Lung Fluid Regulation

The importance of the β_2 AR in lung fluid clearance has been highlighted in several key previous studies. For example, over-expression of the β_2 AR results in lung fluid clearance in animals (Dumasius et al., 2001; Factor et al., 2002). Interestingly, it is through a surge of catecholamines (and presumably stimulation of adrenergic receptors) that results in a clearance of lung fluid following birth in mammals (Finley et al., 1998). In addition, stimulation of the β_2 AR, using salmeterol, results in prevention of high-altitude pulmonary edema in humans and infusion of terbutaline enhances alveolar fluid clearance in healthy lungs and in models of acute respiratory distress syndrome (Lasnier et al., 1996; Sartori et al., 2002). It is likely that lung fluid clearance secondary to stimulation of the β_2 AR occurs via two distinct pathways. First, stimulation of the β_2 ARs can clear fluid from the alveolar airspace through the ENaC (Chen et al., 2001; Eaton et al., 2004; Johnson et al., 2006). Stimulation of the β_2 AR increases the number and probability of an open ENaC on type I and type II alveolar cells (Eaton et al., 2004). This activation of ENaC results in Na^+ , and therefore, fluid out of the alveolar airspace. The second pathway by which stimulation of the β_2 ARs can result in lung fluid removal is at the site of the pulmonary lymphatics. Stimulation of the β_2 AR on lymphatic tissue results in an increase lymphatic relaxation and an increase in lung lymph flow (Ikomi et al., 1991; Mahe et al., 1991).

4.3 The Influence of Hypoxia on Lung Fluid Balance

Prolonged exposure to hypoxia seems to result in lung fluid accumulation in most models. However, in agreement with several animal models (Aarseth et al., 1975; Bland et al., 1977; Homik et al., 1988; Sakuma et al., 2001), we have shown that short-term hypoxic exposure, at rest and during exercise causes a reduction in lung water in healthy humans (Snyder et al., 2006a). Although the exact mechanism by which short-term hypoxia reduces lung fluid is not known, the present study implies a role for an increase in the density and activity of the β_2 AR. Previous studies have suggested that hypoxia decreases the activity of the ENaCs on type-II alveolar cells and alveolar fluid clearance). However, the level of hypoxia used in these studies was severe, ranging from 0–10%, averaging approximately 8% F_{IO_2} , and the inhibition of ENaC appears to be completely reversed with catecholamines or catecholamine analogs (Vivona et al., 2001). A recent study by Suzuki et al. demonstrated no difference in alveolar fluid clearance and an increase in the mRNA of ENaC on with 15% O_2 for 48hrs or 10% O_2 for 24 hours (Suzuki et al., 1999). A study by Vivona et al. showed a significant drop in alveolar fluid clearance with 8% O_2 , but also found an increase in the expression of the alpha subunit of the ENaC on type-II alveolar cells and demonstrated a complete normalization of alveolar liquid clearance when hypoxia was coupled with the infusion of terbutaline, highlighting the importance of the β_2 AR on lung fluid clearance with hypoxia, which is a finding that has been replicated by at least one other group (Loeh et al., 2010; Vivona et al., 2001). It is also possible that the severity of hypoxia or the time of exposure

was not severe enough to result in damage to the alveolar epithelium, which seems to be key to lung fluid accumulation in this model (Bai et al., 2010).

Although the decrease in lung water was unexpected in the present study it may be due to the increase in lung lymph flow, as described by previous authors. Levine et al. have shown that lymph flow increased only 10% with normobaric hypoxia in sheep, while Martin et al. found that lymph flow was increased by approximately 40% in dogs when exposed to hypoxia (Levine et al., 1988; Martin et al., 1986). Increases in ventilation also lead to an increase in lung lymph flow (Pearse et al., 2005). And a previous study by our group showed a significant increase in ventilation with exposure to normobaric hypoxia (Snyder et al., 2006a). Additionally, one of the mechanisms by which hypoxia is thought to result in an increase in lung water is through an increase in pulmonary capillary pressures. Three relatively recent studies have demonstrated that exercise during hypoxia (even in trained individuals) does not result in an increase in lung water, although they likely had dramatic increases in pulmonary pressures given high cardiac outputs coupled with hypoxic pulmonary vasoconstriction (Guenette et al., 2007; Hodges et al., 2007; MacNutt et al., 2007). Of importance, three of our subjects (18%) demonstrated radiographic evidence for an increase in lung water with short-term hypoxic exposure which is in agreement with some previous estimates of the percentage of the population that is susceptible to high-altitude pulmonary edema. This is certainly an interesting topic for future, more detailed, studies.

4.4 The Influence of Hypoxia on β Adrenergic Receptor Density

Several studies have sought to determine the influence of both short-term and prolonged hypoxia on β -adrenergic receptor density in animal models and in cells with conflicting results. One of these previous studies determined that exposure to chronic altitude resulted in a decrease in cardiac β -adrenergic receptor density (Voelkel et al., 1981). The decrease in receptor density in this study is thought to be due to prolonged exposure to circulating catecholamines, which are typically elevated with altitude exposure. In a later study, researchers found that the exposure to hypoxia for three and seven days resulted in no change in downstream signaling of β -adrenergic receptors and found that basal cAMP levels in the pulmonary arteries did not change with hypoxia exposure (Shaul et al., 1990). However, isoproterenol-stimulated cAMP activity in the pulmonary arteries was decreased significantly following hypoxic exposure. In addition, this study demonstrated a decrease in β -adrenergic receptor density in the pulmonary arteries. Interestingly, another study has demonstrated an increase in β_1 AR density in neonatal rat cardiac myocytes following exposure to hypoxia for 72 hours (Li et al., 1996). Despite this increase in β_1 AR mRNA, the authors did not demonstrate an increase in downstream signaling. These three previous studies focus on the β -adrenergic receptor density response in the cardiovascular system. Birkrant et al. have demonstrated that chronic hypoxic exposure increases β -adrenergic receptor density in rats lungs (Birkrant et al., 1991). This is an important finding and one that our results seem to confirm, although future studies are certainly warranted.

5. Limitations

In the present study we determined β_2 AR density using radioligand binding on lymphocytes. Although the amount of adrenergic receptors on lymphocytes has been shown to be related to the number of adrenergic receptors in heart tissue (Qing et al., 1997), future studies should employ a more direct assessment of β_2 AR changes on the lung tissue with hypoxic exposure, such as positron emission tomography. The assessment of lung water remains difficult. In the present study we utilized CT scanning, assessment of lung volumes and airflows, and the assessment of lung diffusion to estimate lung water changes in humans. CT scanning has limitations in that it assesses both blood volume changes along with lung water

as a measure of density. As such, we corrected our change in density with the change in pulmonary capillary blood volume as assessed using D_{CO}/D_{NO} . Importantly, along with a decrease in lung tissue volume using CT scanning we also noted an increase in pulmonary function and alveolar-capillary membrane conductance, both of which seem to confirm the CT findings.

It is possible that there was a diuresis overnight that lead to an overall drop in fluid and was reflected as a drop in lung water in the present study. This can happen with withdrawal of sympathetic tone, or through simple recumbancy-induced diuresis. Because we noted an increase in epinephrine and norpinephrine, it is unlikely that there was a decrease in sympathetic activity; however, one cannot rule out the influence of overnight diuresis with recumbancy as a method by which we demonstrated a loss in lung fluid in the present study. Future study is needed using a normoxic control to determine this.

Also of importance is that all measures of D_{CO} , D_{NO} , and components were made in the upright position, while CT-scanning was performed in the supine position. This was done the same way both at baseline and post-hypoxia. Future studies should consider performing the D_{CO}/D_{NO} maneuver in the supine position when using V_c to estimate lung water from CT-derived lung tissue volume. In addition, training volume, age, stress, alcohol consumption and other lifestyle variables can influence β_2AR density. Although we instructed the subjects to abstain from exercise within 48h of the study, we did not assess training status. However, because this study included a within-subject comparison, we feel that this likely did not influence the results significantly.

6. Conclusion

This study confirms previous studies that have demonstrated that the exposure to short-term normobaric hypoxia results in a decrease in lung water in healthy humans. We add to this previous finding by finding a mechanism by which this decrease in lung water may be happening, through the β_2AR pathway. The increase in the number of β_2AR s on lymphocytes with hypoxia was associated with the loss of lung water and may suggest a role in lung fluid clearance mechanisms in healthy humans. This has important implications in conditions where lung fluid accumulation is likely.

Acknowledgments

This work was supported by NIH Grant HL71478. We would like to thank Kathy O'Malley, for her help with data collection and Renee Blumers for her help with manuscript preparation as well as the efforts of the study participants. We would also like to thank the staff of the Center for Translational Sciences Activities (CTSA) for their assistance throughout this study. The Mayo Clinic CTSA is supported by US Public Health Service grant M01-RR00585.

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Highlights

- > We explored the influence of hypoxia on lung fluid changes and how these changes were related to changes in beta-2 adrenergic receptor density.
- > We found a reduction in lung water (using CT scanning) and an improvement in lung diffusion.
- > We found that these changes in indices of lung water were related to changes in beta-adrenergic receptor density.

Figure 1.

Change in pulmonary function with 17-hours of hypoxic exposure. FVC=forced vital capacity, FEV₁=forced expiratory volume after one second, FEF₂₅₋₇₅=forced expiratory flow at 25–75% of the FVC. The x-axis represents pulmonary function measures and the y axis represents liters (for FVC and FEV₁) and liters per second for FEF₂₅₋₇₅. *p<0.05 when compared to normoxia.

Figure 2.

Change in lymphocyte β_2 -adrenergic receptor density with exposure to 17-hours of normobaric hypoxia. The filled column represents the pre-hypoxia receptor density and the unfilled column represents the post-hypoxia receptor density. * $p < 0.05$ when compared to normoxia.

Figure 3. Individual changes in computed tomography-derived lung tissue volume with 17-hours of hypoxic exposure. The x-axis represents the condition and the y-axis represents the tissue volume, as determined with CT-scanning, in milliliters. *represents the mean for each condition.

Figure 4.

Individual changes in lung water following 17-hours of hypoxic exposure. The x-axis represents subject number and the y-axis represents the change in lung water (determined through the mathematical correction of lung tissue volume changes with changes in pulmonary capillary blood volume).

Figure 5.

Relationship between the changes in lymphocyte β_2 -adrenergic receptor density and lung water with 17-hours of hypoxic exposure. The x-axis represents the change in β_2 -adrenergic receptor density on lymphocytes and the y-axis represents the change in lung water, in milliliters. $P < 0.01$.

Table 1

Subject Characteristics.

n (%female)	13(34)
Age (years)	31±3
Height (cm)	179±3
Weight (kg)	78±3
BMI (kg/m²)	24±1
VO_{2peak} (ml/kg/min)	40±2

BMI=body mass index, VO_{2peak}=maximal oxygen consumption (as determined with cycle ergometry test).

Table 2

Physiological Adjustments to 17 hr Normobaric Hypoxia

Variable	Normoxia	Hypoxia
SaO ₂ (%)	97.5±0.3	82.5±0.9 *
RVSP (mmHg)	13.9±2.6	29.4±2.2 *
HR (bpm)	61±2	75±3 *
MAP (mmHg)	89±2	88±2
SVR (dynes×sec/cm ⁵)	1431±97	1471±91
Q (L/min)	5.2±0.2	5.0±0.2
EPI (ng/100mL)	16.9±4	18.2±4 *
NE (ng/100mL)	185±26	221±35 *
β ₂ AR (receptors/lymphocyte)	1497±187	1850±225 *
Symptoms (LLS)	0±0	1.1±0.2 *
D _M (ml/mmHg/min)	34±2	46±3 *
V _c (ml)	93±12	118±21 *
D _M /V _c	0.36±0.10	0.47±0.10

Values are mean±SEM. SaO₂=peripheral oxygen saturation, RVSP=right ventricular systolic pressure, HR=heart rate, MAP=mean arterial blood pressure, SVR=systemic vascular resistance, Q=cardiac output, EPI=epinephrine, NE=norepinephrine, LLS=Lake Louise Score (modified), D_M=alveolar-capillary membrane conductance, V_c=pulmonary capillary blood volume, D_M/V_c=alveolar-capillary membrane conductance corrected for pulmonary capillary blood volume.

* Hypoxia significantly different from normoxia p<0.05